SKIN TESTING FOR INHALANT ALLERGY 2003: CURRENT STRATEGIES

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Table 3. Sample titration with optimized IDT (EP in parenthesis)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. 6</th>
<th>No. 5</th>
<th>No. 4</th>
<th>No. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bermuda</td>
<td>5</td>
<td>(*)</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Timothy</td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ragweed</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamb’s quarters</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsh elder</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oak</td>
<td>5</td>
<td>5</td>
<td>(*)</td>
<td></td>
</tr>
<tr>
<td>Elm</td>
<td>5</td>
<td></td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>Mountain cedar</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternaria</td>
<td>(*)</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Cladosporium</td>
<td></td>
<td></td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Helminthosporium</td>
<td>(*)</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>D farinae</td>
<td></td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Diluent</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerine No. 2</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine No. 3</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Titration performed in spring, when grass and trees are in season, so these are started at No. 6. EP indicated by an asterisk is estimated based on wheal size at stronger dilution. Note that all EPs are the same as in the previous table except for cat, which is started one dilution weaker than true EP. The total number of sticks, including controls, was 19.

Positive “endpoint” wheal grows to 7 mm or more and that testing with one concentration stronger than the one that produced the endpoint response generally yields a wheal of 9 mm diameter or greater, it is possible to extrapolate and “fill in the blanks” of a titration that does not necessarily involve placing a skin test with the use of every possible dilution (Table 3).

For example, if testing a patient who does not fit the criteria noted above of a “brittle” patient, one could apply a No. 4 dilution. If the wheal produced grew to 9 mm, extrapolation would suggest that the endpoint was at No. 5. This could be confirmed, if desired, by placing a No. 5 dilution to produce a wheal of approximately 7 mm in diameter. If testing at a No. 4 dilution produced a negative wheal, a test at No. 2 strength would be applied. If the wheal produced by the No. 2 dilution grew to 7 mm in diameter, one could assume that the endpoint was at No. 2, whereas a negative wheal at this concentration would indicate a negative titration for this antigen. Although the placement of a confirmatory wheal was suggested by Rinkel to verify the specific endpoint of titration and to reduce the incidence of false-positive reactions derived from single dilutional intradermal testing, in most cases it is not absolutely necessary. Testing based on the classification of a 7-mm wheal as positive without a confirmatory wheal will rarely yield results that differ significantly from those obtained through requiring a 9-mm confirmatory wheal. Furthermore, this approach will not adversely affect the safe provision of immunotherapy. If the skilled clinician elects to eliminate the confirmatory wheal from the IDT battery, it will decrease both the number of needle sticks and the time necessary to complete the testing.

With the use of a 14-antigen screen and testing in the fashion described above, it is possible to confirm a negative titration with no more than 30 sticks, including controls. Additional efficiency may be achieved by combining this optimized IDT technique with other methodologies.

**Benefits of intradermal titration.** The quantitative methodology of IDT allows identification of both low and high degrees of sensitivity and provides information about a safe starting dose for immunotherapy in both instances. The antigens included in the treatment set are generally taken from the stock vials used to prepare the material from which the testing was done, so each patient’s response to them has already been assessed by a bioassay. Although sensitivities change with antigen exposure, IDT assesses the patient’s current degree of sensitivity, either in or out of season. Although circumstances and constraints may make it impractical to perform a full titration on every patient, the principles of quantitative testing and development of a treatment regimen tailored to each individual patient remain central to the practice of otolaryngic allergy.

**Modified Quantitative Testing**

As demonstrated above, IDT offers a thorough, comprehensive, quantitative approach to skin testing for inhalant allergy. It is safe, because it progresses gradually from weaker to more concentrated antigenic solutions, at the same time permitting precise, quantifiable measurement of allergenic sensitivities. The increased commitment in testing time and costs that is associated with clas-
sic SET is partially offset by using the optimized form of IDT just described.

Recognizing that quantification is important in determining safe and effective starting doses for immunotherapy, but also considering the time and financial constraints of IDT, methods have more recently been sought to modify the testing method described above while maintaining its ability to discriminate quantitatively. Several approaches have been developed in order to reach an appropriate compromise between efficiency and accuracy. One such approach is modified quantitative testing (MQT). This technique uses prick testing initially to determine an approximate range of sensitivity, with a single weaker or more concentrated intradermal test used to define the level of sensitivity and quantify the allergic response.

One consideration that is important in the use of prick testing techniques is that not all prick testing methods or devices are equivalent. Some prick testing devices provide more replicable results than others, and the sensitivity and specificity of testing with these various devices vary.\textsuperscript{38,39} In general, those prick devices that allow reproducible delivery of a standard amount of antigen to a uniform depth within the epidermis are preferable to those devices that are less reproducible. The best current prick instruments are multipronged devices that do deliver a reproducible amount of antigen to a controlled depth. These devices have been shown to be less technician-dependent than the Morrow-Brown needle, with results that are therefore more reliable and reproducible.\textsuperscript{40} With these multipronged devices, several antigens can be placed on the skin simultaneously, with precision in both the amount of antigen that is delivered and the depth of delivery. These multipronged devices have accompanying testing wells that contain antigen concentrate into which the devices are placed before testing. They also have multiple fine tines in which a precise amount of antigen concentrate is held by capillary action.

The device that was used in MQT as originally described is the Multi-Test II device (Lincoln Diagnostics, Inc, Decatur, IL), although other devices, such as the Quintest device (Hollister-Stier Laboratories, LLC, Spokane, WA) may be used as well.

\textbf{Fig 6. Placement of antigens into forearm with Multi-Test device.}

\textbf{Technique of MQT.} The patient is brought into the testing room and properly identified. The patient is then questioned concerning the use of any medications that may adversely affect either the accuracy or the safety of the test. These medications include β-blockers, tricyclic antidepressants, and antihistamines. Both the volar surface of the forearms and the upper outer aspects of both arms are used for testing, so these areas are exposed. The forearms are first cleaned with alcohol, and ink is used to mark the location and orientation of the testing punctures that will be placed.

The antigens to be tested have been previously positioned in testing wells in a discrete pattern so that they can be identified after they have been placed on the skin. Both a positive (histamine) control and a negative (saline solution) control are included in the testing board. If there is any question about the skin’s ability to respond to testing (as with a patient in whom current histamine blockade is unknown), an individual prick test with histamine may be placed before full testing. The test antigens are then placed onto the forearm with moderate pressure and a gentle rocking motion both from side to side and from forward to backward (Fig 6). As the device is taken off the skin, small droplets of antigen will remain at the individual testing sites and should not be wiped clean for at least 5 minutes. Patients should be advised to keep their arms relatively immobile during those 5 minutes to prevent cross-contamination of antigens. For screening purposes, 1 Multi-Test II device of 8 tests may be placed on each volar forearm. For any additional testing, up to 2 panels of 8 tests each, may be placed on each
volar forearm, for a total of 30 antigens and 2 controls. (In young children there may not be adequate surface area to allow use of 2 devices on each forearm, and testing may need to be conducted on either the anterior thigh or the back.)

After placement of the tests, 20 minutes are allowed for the wheals to develop at the site of each test. The wheals are then measured and their size recorded. A positive test is defined as a wheal with a diameter of 3 mm or greater at 20 minutes, consistent with the guidelines of the European grading systems (Fig 7). If all tests show whealing, as may occur with a dermatographic patient or with a patient who is overly sensitive to the trauma of the puncture, a positive wheal is defined as a wheal with a diameter of 3 mm or greater than the diameter of the negative control wheal. All positive test responses are then circled on the recording sheet for ease of identification.

On the basis of whether the prick test is negative, a single intradermal test of either a No. 2 dilution (1:500 wt/vol) or a No. 5 dilution (1:62,500 wt/vol) is then placed on the upper outer arm. The previous experience of Murphree and Kniker with this testing technique suggests that the level of response to the Multi-Test device approximates that of a 1:1,500 wt/vol intradermal test, approximately corresponding to a No. 3 dilution SET test. MQT is designed to further refine this initial estimate of sensitivity through use of intradermal tests with either stronger or weaker concentrations of antigens. These intradermal tests can then be used to estimate dilutions of antigen with an IDT model and therefore to provide a quantitative basis for the provision of immunotherapy.

In practice, once the prick testing results are recorded, a single intradermal test is then applied for each of the antigens tested. If the prick response for a specific antigen is negative (<3 mm wheal), a single 4-mm intradermal wheal with a No. 2 dilution of that antigen is applied to the upper outer arm. If the prick response is positive (≥3 mm wheal), a single 4-mm intradermal wheal with a No. 5 dilution of that antigen is applied to the upper outer arm. A positive response to intradermal testing is a wheal of 7 mm or greater in diameter at 10 minutes. On the basis of the results of these intradermal tests, an approximate endpoint can be determined that can be used to prepare treatment vials for immunotherapy. Application of these results is noted in the MQT algorithm shown in Fig 8. The one exception to this two-stage procedure is in the case of a large response to the initial prick test. By definition, a response of 9 mm or greater in whealing to the initial prick test would be assigned an endpoint value of No. 6, and no intradermal test would be placed.

By reference to the algorithm (Fig 8), a response to an individual antigen that is negative to both the initial prick test and to the No. 2 intradermal test is classified as a negative response to that antigen. A response to an individual antigen that is negative to the initial prick test but positive to the No. 2 intradermal test is classified as an endpoint of No. 3. A response to an individual antigen that is positive to the initial prick test and negative to the No. 5 intradermal test is classified as an endpoint of No. 4. A response to an individual antigen that is positive to the initial prick test and positive to the No. 5 intradermal test with a wheal size of 7 or 8 mm is classified as an endpoint of No. 5. A response to an individual antigen that is positive to the initial prick test and positive to the No. 5 intradermal test with a wheal size of 9 mm or greater is classified as an endpoint of No. 6. These endpoints are considered to correspond to the endpoints obtained with a full, standard SET battery with the use of several sequential intradermal tests, and dose calculation and vial preparation for immunotherapy are approached in the same manner.
Because MQT uses IDT as the final step in quantifying the patient's sensitivity, it can appropriately be classed as one form of IDT.

**Prick/Puncture Testing**

Epicutaneous tests were among the earliest tests used for the diagnosis of inhalant allergy. These techniques can be divided into scratch tests, in which a superficial cut is made into the epidermis and antigen is applied to the denuded skin, and prick/puncture tests, in which a drop of antigen is introduced into the epidermis through a superficial needle prick or puncture. Scratch testing, which has been noted to have both poor sensitivity and poor specificity, is mentioned here for historical purposes only. Because of its poor clinimetric properties and the availability of more accurate techniques, the AMA Council on Scientific Affairs long ago recommended against the use of this outmoded technique.43

For the purposes of this monograph, all prick/puncture techniques, whatever device is used for their application, will be generically referred to as prick tests. Prick testing is in routine use throughout the world, often without intradermal testing, for the diagnosis of allergy and the provision of immunotherapy. This therapy, however, involves empiric dosing, rather than being based on quantitative information such as is available through IDT. Several specific methods have been described for conducting prick tests. Single-antigen prick devices include lancets, the Morrow-Brown needle (Antigen Laboratories, Inc, Liberty, MO), and the DuoTip device (Lincoln Diagnostics, Inc).

For all types of prick testing (except those which use a multiple prick applicator, the technique for which has already been described), the patient is first brought into the testing room and properly identified. The patient is then questioned concerning the use of any medications that may adversely affect either the accuracy or the safety of the test, including β-blockers and antihistamines. Testing may be done on the upper back, the volar surface of the forearms, and/or the upper outer aspects of both arms. The physician must be aware that not all anatomic sites demonstrate the same degree of skin reactivity. In general, the most brisk skin reactions will be found on the upper back, which is why this site was often chosen for skin testing when scratch techniques were used. In decreasing order, the degrees of reactivity are as follows: mid and upper back > lower back > upper arm > elbow > forearm (ulnar > radial) > wrist.44 These areas are exposed as indicated. The skin is first cleaned with alcohol, and ink is used to mark the location and orientation of the testing pricks that will be placed.

With the lancet devices, a small drop of antigen concentrate (1:20 wt/vol or standardized antigen
concentrate) is placed onto the skin and this solid needle is then used to elevate or "tent" the skin through the drop of antigen, taking care not to penetrate into the dermis. The Morrow-Brown needle can also be used to introduce antigen into the skin. With this technique the needle is placed through the antigen drop perpendicular to the skin. The depth of penetration is controlled by a small flange on the shaft of the needle. Several grading systems are available for evaluating the response, ranging from a 1+ to 4+ system in which both wheal and flare (erythema) are used to grade the response, to measuring and recording the size of the wheal to reflect the degree of reactivity. These responses are read for each individual antigen at 10 minutes after placement. The technique is subject to some variation of depth of penetration, and thus the intensity of the wheal-and-flare reaction may also vary. For that reason, lancet-based techniques have grown less popular.

To improve the reliable and objective measurement of testing results, the use of wheal measurements for assessment of skin reactivity is preferred. The degree of erythema (flare) is considered to be a nonspecific reaction of the skin to the trauma of the puncture and should not be used in the assessment or measurement of the degree of antigen-specific sensitivity. A wheal that grows to a diameter of ≥3 mm greater than a negative control is judged to be positive. The size of the wheal is recorded for each antigen that is tested.

Once prick testing has been completed for all antigens, the testing results are used to determine the contents of the treatment vial if immunotherapy is contemplated. Prick tests, as a group, lack the degree of quantitative information that is available with either IDT or MQT. Given that only the whealing response to one discrete concentration of antigen has been assessed, it can be difficult to prepare a treatment vial that will be potent enough to provide efficient escalation of immunotherapy doses yet maintain the degree of safety inherent in quantitative testing approaches. This factor is the major issue that is of concern in delivering immunotherapy on the basis of prick testing alone.

Despite this caution, it is possible to deliver immunotherapy on the basis of a single prick test with each antigen. This practice is common in both Europe and the United States, even among clinicians who use single intradermal tests in addition to prick tests. Several considerations are important in considering immunotherapy based on prick testing. First, the standard method by which many allergists use prick-based immunotherapy is through preparing treatment vials with all antigens to which a patient has reacted at extremely dilute concentrations, such as 1:1,000,000 wt/vol. Although these dilutions will likely be safe, this approach will require many months before the antigen levels are adequate to promote an immune response that will result in symptomatic improvement. In addition, because the degrees of sensitivity to various antigens will likely differ among antigens, preparation of immunotherapy vials with uniform concentrations of antigens will tend to result in adverse local and systemic reactions to some antigens that will limit even a minimal therapeutic response to other antigens. It is this observation that has guided otolaryngic allergy practice from the outset and led early clinicians such as Hansel and Rinkel to develop SET.

To achieve a very rough quantification of testing results, some practitioners divide testing results into two or more categories of response levels—high- and low-sensitivity responses—on the basis of wheal size. They then use two different dilutions of antigen in preparation of immunotherapy vials. One such approach involves dividing
positive responses into (1) low-level responses, with wheal sizes of $\geq 3$ mm but $\leq 8$ mm, and (2) high-level responses, with wheal sizes of $\geq 9$ mm. The assumption here is that the degree of responsiveness on the skin provides an estimate of the amount of antigen that is necessary to provoke a certain level of response. With this two-level system, two different dilutions of antigens can then be used in vial preparation. In the first group, the low-level antigen responses, a conservative estimate would be to classify these as positive at IDT dilution No. 4, or 1:12,500 wt/vol. The second group, the high-level responses, would then be classified conservatively as positive at an IDT dilution No. 6, or 1:312,500 wt/vol. It must be emphasized that this approach is semiquantitative and does not provide the precision (or safety) achieved with the quantitative methods already described. Although immunotherapy vials for use in desensitization are often prepared from prick testing results alone by experienced allergists, this technique should only be used with extreme caution by novices or inexperienced physicians.

Second, the provision of safe immunotherapy relies on the use of a vial test before treatment with the initial set of immunotherapy vials. The use of a vial test is strongly recommended with IDT and is mandatory with MQT and all prick testing, as it is with in vitro testing. The vial test involves the raising of a 4-mm intradermal wheal with each immunotherapy vial that has been prepared. If the wheal grows to greater than 13 mm in diameter at 10 minutes, the vial is overly concentrated and must be diluted 5-fold before beginning immunotherapy. If the wheal is less than 13 mm in diameter at 10 minutes, immunotherapy can be initiated at that time, and if the wheal is precisely 13 mm, the initiation of immunotherapy is deferred until the following treatment visit, at least 72 hours later than the time of the vial test.

With care to detail and with the added safety of the vial test, immunotherapy based on prick testing alone can be safe and effective in the treatment of inhalant allergy. When patients do not respond to immunotherapy conducted with prick testing alone after a reasonable period of time (3-6 months), it would be prudent to consider retesting the patient with a more thorough, quantitative approach in order to assess whether some low-level sensitivities may have been missed with prick testing. Consideration of full IDT or intradermal testing based on the MQT algorithm (Fig 8) and the results of the initial prick testing would be acceptable in these patients.

**SUMMARY**

The otolaryngic allergist has a variety of skin testing methods available for the diagnosis of inhalant allergy. The precise technique or techniques that each physician will select will depend on his or her individual practice characteristics and his or her familiarity with and confidence in those available methods. The AAOA, in its 2003 principles for the diagnosis of inhalant allergy, has recognized that there is no one method that will meet the needs of all patients or physicians. There is little question that a full SET titration provides the most information of any type of skin test, but because of various factors (some, unfortunately, socioeconomic), it is rarely feasible. With the use of an optimized approach through IDT, information may be gained that will often suffice. Screening with prick tests and then achieving quantification through MQT is another effective alternative. Treating from prick test results alone may be appropriate in the hands of experienced practitioners, although novices should realize its lack of precise quantification. No one method will be applicable in every circumstance, and the otolaryngic allergist should strive for familiarity with all techniques. As is generally noted in the practice of medicine, it is the flexible application of many techniques chosen for use in individual circumstances with individual patients that allows the successful treatment of complex medical illnesses.

Otolaryngic allergy cannot be practiced by rote and flow sheet. In order to practice otolaryngic allergy safely and effectively, it is important for every otolaryngic allergist to understand the science of immunology as it relates to IgE-mediated hypersensitivity, the relevant pharmacology of agents used to treat these illnesses, and the anatomy and physiology of the nose and paranasal sinuses. The practice of otolaryngic allergy can at times be more an art than a strict science, but without a thorough working knowledge of the relevant basic and clinical sciences underlying its
practice, the physician will likely experience suboptimal treatment outcomes for his or her patients.

This monograph has been designed to summarize the available types of skin testing concisely so that otolaryngic allergists may use them in the management of their patients. It is not meant to be a substitute for the excellent journal articles and textbooks that discuss this topic in greater depth. It is hoped that it will serve as a springboard for additional reading and study. The practice of otolaryngic allergy is fluid and dynamic, and it is necessary for each of us to continue to study, learn, and critically analyze our methods and our knowledge base for the benefit of the specialty and, more importantly, for the health and welfare of our patients.

REFERENCES